might mediate guidance of POC axons in response to positive cues from Slit1a. Together these data support a model in which distinct combinations of Slit-Robo signaling are required for the differential guidance of and interactions between commissural axons and astroglial cells.

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Program/Abstract # 192
The fasciculation of spiral ganglion peripheral axons in the mouse cochlea is dependent on Pou3F4
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During the formation of the cochlea, spiral ganglion neurons (SGNs) delaminate from the otocyst, and then project peripheral axons into the cochlear epithelium to innervate inner and outer hair cells. During this process, the SGNs form regularly spaced arrays of fascicles that are partitioned from one another by bands of otic mesenchymal cells along the length of the cochlear duct — events thought to be important for normal cochlear function. In the present study, we demonstrate that the expression of the transcription factor Pou3F4 is restricted to the otic mesenchyme (and is not expressed by neurons) during periods of SGN delamination and outgrowth. SGNs from mice that lack Pou3F4 form correctly, but their axons fail to properly fasciculate, and aberrantly invade the adjacent mesenchyme. Similarly, Schwann cells that normally reside proximal to the ganglion appear to disperse among mesenchyme cells that lack Pou3F4. To explore this interaction further, we developed an in vitro assay in which SGNs and otic mesenchyme cells may be co-cultured in the presence of antisense Morpholinos. In these experiments, depleting Pou3F4 substantially reduced the diameter of SGN axons bundles and caused a more disorganized pattern of outgrowth. These data suggest that Pou3F4-positive mesenchymal cells promote SGN fasciculation through the production of inhibitory or repulsive signals. The nature of these signals is unknown, but we are conducting microarray expression screens to identify chemorepulsive signals in mesenchymal cells that may be regulated by Pou3F4.

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Program/Abstract # 193
Regulation of endodermal cell migration by Rac1 during zebrafish gastrulation
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Intrinsic cell migration can either be directionally persistent, migrating in the same direction over long periods of time, or directionally random, frequently making turns and changing direction. Different cell types exhibit different modes of intrinsic migration, and the same cell can switch migration modes at different developmental timepoints. However, the significance of these differences is poorly understood. Recent studies in zebrafish have suggested that during early phases of gastrulation, endodermal cells exhibit rapid, directionally random migration. In order to understand the mechanisms underlying endodermal cell motility during gastrulation, we have generated a transgenic line in which the endoderm-specific sox17 promoter drives expression of a fluorescent actin probe consisting of the actin-binding domain of Utrophin fused to GFP (Tg (sox17: GFP-Utr)). Time-lapse imaging revealed that migratory endodermal cells often exhibit multiple areas of high actin content that appear to be correlated with the ability of these cells to change direction quickly and often. The Rho GTPase Rac1 promotes actin polymerization, and in vitro studies have shown that Rac1 is sufficient to induce directionally random migration. Indeed, when we expressed low amounts of dominant-negative Rac1, we observed a significant increase in directional persistence of endodermal cells. We are currently performing transplantation experiments to test whether low Rac1 activity affects the ability of cells to contribute to the endoderm. We are also exploring whether endoderm-promoting signals such as Nodal can regulate Rac1 activity.

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Program/Abstract # 194

ABSTRACT WITHDRAWN

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Program/Abstract # 195
Functional analysis of methylation during neural crest migration
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Neural crest cells are multipotent, migratory cells that arise from the vertebrate dorsal neural tube, migrate extensively, and form diverse lineages including the craniofacial bone, pigment cells, and the peripheral nervous system. Delamination of neural crest cells from the dorsal neural tube occurs over an extended period of time, indicating that cells are not selected to migrate all at once. It is not known how the activity of migration-related proteins is temporally controlled to produce motile neural crest cells. Analysis of methylation in neural crest cells suggests that post-translational methylation of cytoplasmic, non-histone proteins regulates migration. Mono- and di-methylated lysine is localized and elevated in the cytoplasm of neural crest cells as they arise from the dorsal neural tube and initiate migration, supporting a role for methylated cytoplasmic proteins in neural crest cell migration. Ezh2 is a candidate methyltransferase for the methylation of cytoplasmic proteins. A dominant-negative and cytoplasmically localized Ezh2 construct is being used to specifically characterize Ezh2's role in cytoplasmic protein methylation during neural crest development. In addition, efforts are underway to profile cytoplasmic and nuclear methylated proteins in premigratory and migratory neural crest cells. Cytoplasmic protein methylation is a novel way to think about how proteins are regulated. Establishing a functional role for methylation in neural crest migration will provide insight into the molecular mechanisms of related processes such as cancer metastasis. Funded by F31 DE019755, March of Dimes, and a U of MN AHC Seed Grant.

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